

Apple. No. 09/863,693

Amendment dated March 30, 2004

Reply to Office Action of March 27, 2003 and Notice of Non-Compliant Amendment dated March 16, 2004

This listing of claims will replace all prior versions and listings of claims in the application:

**Listing of Claims:**

1-29. canceled

30. (previously presented) A method of preparing a bispecific antibody comprising a first polypeptide and a second polypeptide, wherein

(a) the first polypeptide comprises a first multimerization domain that interacts with a multimerization domain of the second polypeptide,

(b) the first polypeptide and second polypeptide each comprise a different binding domain, a first binding domain comprising a first antibody variable heavy chain and an antibody variable light chain, and a second binding domain comprising a second antibody variable heavy chain and said antibody variable light chain, and

(c) the bispecific antibody is formed by said variable light chain interacting with the first variable heavy chain in the first binding domain, and said variable light chain interacting with the second variable heavy chain in the second binding domain, the method comprising the steps of:

(i) culturing a host cell comprising nucleic acid encoding the first polypeptide and second polypeptide, and said variable light chain, wherein the culturing is such that the nucleic acid is expressed; and

(ii) recovering the bispecific antibody from the host cell culture.

31. (previously presented) The method of claim 30, wherein the first polypeptide and second polypeptide each comprise an antibody constant domain.

32. (previously presented) The method of claim 31, wherein the first polypeptide and second polypeptide each comprise an antibody constant domain from a C<sub>H</sub>3 domain or from an IgG.

Apple. No. 09/863,693

Amendment dated March 30, 2004

Reply to Office Action of March 27, 2003 and Notice of Non-Compliant Amendment dated March 16, 2004

33. (currently amended) A method of preparing a bispecific antibody comprising a first polypeptide and a second polypeptide, wherein

(a) the first polypeptide comprises a first multimerization domain that interacts with a multimerization domain of the second polypeptide,

(b) the first polypeptide and second polypeptide each comprise a different binding domain, a first binding domain comprising a first antibody variable heavy chain and a first antibody variable light chain having at least one three CDRs, and a second binding domain comprising a second antibody variable heavy chain and a second antibody variable light chain having at least one three CDRs, wherein said at least one CDR of the first and second variable light chains have a common amino acid sequence at least 98% sequence identity and only differ at amino acid positions outside of the CDRs, and

(c) the bispecific antibody is formed by the first variable light chain interacting with the first or second variable heavy chain in the first or second binding domain, and the second variable light chain interacting with the first or second variable heavy chain in the first or second binding domain, the method comprising the steps of:

- (i) culturing a host cell comprising nucleic acid encoding the first polypeptide and second polypeptide, and the first and second variable light chain, wherein the culturing is such that the nucleic acid is expressed; and
- (ii) recovering the bispecific antibody from the host cell culture.

34. (previously presented) The method of claim 33, wherein the first polypeptide and second polypeptide each comprise an antibody constant domain.

35. (previously presented) The method of claim 34, wherein the first polypeptide and second polypeptide each comprise an antibody constant domain from a C<sub>H</sub>3 domain or from an IgG.

Apple. No. 09/863,693

Amendment dated March 30, 2004

Reply to Office Action of March 27, 2003 and Notice of Non-Compliant Amendment dated March 16, 2004

36. (currently amended) A method of preparing a bispecific antibody comprising a first polypeptide and a second polypeptide, wherein

(a) the first polypeptide comprises a first multimerization domain that interacts with a multimerization domain of the second polypeptide, wherein each of the multimerization domains comprises a residue with a free thiol positioned so that a disulfide bond is formed between the first and second polypeptides,

(b) the first polypeptide and second polypeptide each comprise a different binding domain, a first binding domain comprising a first antibody variable heavy chain and a first antibody variable light chain having three CDRs, and a second binding domain comprising a second antibody variable heavy chain and a second antibody variable light chain having three CDRs, wherein the first and second variable light chains have at least 80% 98% sequence identity, and only differ at amino acid positions outside of the CDRs; and

(c) the bispecific antibody is formed by the first variable light chain interacting with the first or second variable heavy chain in the first or second binding domain, and the second variable light chain interacting with the first or second variable heavy chain in the first or second binding domain, the method comprising the steps of:

- (i) culturing a host cell comprising nucleic acid encoding the first polypeptide and second polypeptide, and the first and second variable light chain, wherein the culturing is such that the nucleic acid is expressed; and
- (ii) recovering the bispecific antibody from the host cell culture.

37. (previously presented) The method of claim 36, wherein the first polypeptide and second polypeptide each comprise an antibody constant domain.

38. (previously presented) The method of claim 37, wherein the first polypeptide and second polypeptide each comprise an antibody constant domain from a C<sub>H</sub>3 domain or from an IgG.

Apple. No. 09/863,693

Amendment dated March 30, 2004

Reply to Office Action of March 27, 2003 and Notice of Non-Compliant Amendment dated March 16, 2004

39. (previously presented) A host cell comprising nucleic acid encoding a bispecific antibody comprising a first polypeptide and a second polypeptide, wherein

(a) the first polypeptide comprises a first multimerization domain that interacts with a multimerization domain of the second polypeptide,

(b) the first polypeptide and second polypeptide each comprise a different binding domain, a first binding domain comprising a first antibody variable heavy chain and an antibody variable light chain, and a second binding domain comprising a second antibody variable heavy chain and said antibody variable light chain, and

(c) the bispecific antibody is formed by said variable light chain interacting with the first and second variable heavy chain in the first and second binding domain.

40. (previously presented) The host cell of claim 39 wherein the host cell is a mammalian cell.

41. (currently amended) A host cell comprising nucleic acid encoding a bispecific antibody comprising a first polypeptide and a second polypeptide, wherein

(a) the first polypeptide comprises a first multimerization domain that interacts with a multimerization domain of the second polypeptide,

(b) the first polypeptide and second polypeptide each comprise a different binding domain, a first binding domain comprising a first antibody variable heavy chain and a first antibody variable light chain having ~~at least one~~ three CDRs, and a second binding domain comprising a second antibody variable heavy chain and a second antibody variable light chain having ~~at least one~~ three CDRs, wherein said ~~at least one CDR of the first and second variable light chains have a common amino acid sequence at least 98% sequence identity and only differ at amino acid positions outside of the CDRs.~~ and

(c) the bispecific antibody is formed by the first variable light chain interacting with the first or second variable heavy chain in the first or second binding

Apple. No. 09/863,693

Amendment dated March 30, 2004

Reply to Office Action of March 27, 2003 and Notice of Non-Compliant Amendment dated March 16, 2004

domain, and the second variable light chain interacting with the first or second variable heavy chain cell culture.

42. (previously presented) The host cell of claim 41 wherein the host cell is a mammalian cell.

43. (currently amended) A host cell comprising a nucleic acid encoding a bispecific antibody comprising a first polypeptide and a second polypeptide, wherein

(a) the first polypeptide comprises a first multimerization domain that interacts with a multimerization domain of the second polypeptide, wherein the each of the multimerization domains comprises a residue with a free thiol positioned so that a disulfide bond is formed between the first and second polypeptides,

(b) the first polypeptide and second polypeptide each comprise a different binding domain, a first binding domain comprising a first antibody variable heavy chain and a first antibody variable light chain having three CDRs, and a second binding domain comprising a second antibody variable heavy chain and a second antibody variable light chain having three CDRs, wherein the first and second variable light chains have at least 80% 98% sequence identity, and only differ at amino acid positions outside of the CDRs; and

(c) the bispecific antibody is formed by the first variable light chain interacting with the first or second variable heavy chain in the first or second binding domain, and the second variable light chain interacting with the first or second variable heavy chain in the first or second binding domain.

44. (previously presented) The host cell of claim 43 wherein the host cell is a mammalian cell.

Applic. No. 09/863,693

Amendment dated March 30, 2004

Reply to Office Action of March 27, 2003 and Notice of Non-Compliant Amendment dated March 16, 2004

45. (new) The method of claim 30, wherein the antibody variable light chain is identical to an original antibody variable light chain of the first and second polypeptide.

46. (new) The method of claim 30, wherein the antibody variable light chain has at least about 98% sequence identity to an original antibody variable light chain of the first and second polypeptide.

47. (new) A method of preparing a bispecific antibody comprising:

- (a) selecting a variable light chain domain that has at least 98% sequence identity to each variable light chain domain of a first and second antibody, wherein the first and second antibody bind to different antigens;
- (b) culturing a host cell comprising:
  - (i) a first nucleic acid sequence encoding a first variable heavy chain domain from the first antibody and a first multimerization domain;
  - (ii) a second nucleic acid encoding a second variable heavy chain domain from the second antibody and a second multimerization domain; and
  - (iii) a third nucleic acid encoding the selected variable light chain domain;wherein the first variable heavy chain variable domain and the variable light chain domain form a first binding site for a first antigen and the second variable heavy chain domain and the variable light chain form a second binding domain for a second antigen; and the first and second multimerization domains interact to form a bispecific antibody; and
- (c) recovering the bispecific antibody from the cell culture.

48. (new) The method of claim 47, wherein the selected light chain variable domain is 100% identical to each light chain variable domain of the first and second antibody.

Apple. No. 09/863,693

Amendment dated March 30, 2004

Reply to Office Action of March 27, 2003 and Notice of Non-Compliant Amendment dated March 16, 2004

49. (new) The method of claim 47, wherein each of the first and second multimerization domains comprise a C<sub>H</sub>3 domain of an antibody constant domain.

50. (new) The method of claim 49, wherein the first multimerization domain has a protuburance and the second multimerization domain has a cavity and the first and second multimerization domains dimerize by the fitting of the protuburance into the cavity.

51. (new) The method of claim 50, wherein the multimerization domain also comprises a non-naturally occurring disulfide bond.